

Monoclonal antibody against mice IFN alpha - uses rat spleen cell, immunised with IFN antigen, as antibody producing cell

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Abstract:

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Monoclonal antibody has specificity against mice IFN-alpha.

In prepn. mice L cell IFN induced by Newcastle disease virus is used as crude material. Sheep antibody against mice L cell IFN-alpha,beta is immobilised on Affigel 10, and by using thus prepd. antibody affinity column chromatography, high purity IFN-alpha,beta-mixt. (spec. activity, 10 power 8 IU/mg protein) is obtd., and used as antigen.

To ACI rats (age 8 weeks) the mice IFN-alpha,beta mixt. is administered with Freund's complete adjuvant, each 10 power 7 IU at 2 weeks interval for 3 times. After further 4 weeks, equal amt. of IFN antigen without adjuvant is injected (i.p).

As antibody producing cell, rat's spleen cell, after 3rd day of final immunisation, is used. In prepn. of myeloma cell. P3-X63-Ag8-6.5.3 is cultured on 10% FCS contg. RPMI 1640 medium. Above myeloma cell and rats spleen cell are mixed at 1:3, and fused by PEG, and cultured on HAT medium by 96 hole microplate. Antimice IFN-alpha antibody producing hybridoma is cloned, and monoclonal hybridoma 9B12 strain is obtd. The strain is cultured on 10% FCS contg. RPMI 1640 medium and 10 power 7 cell is washed by PBS, and injected (i.p.) to Balb/c nude mice which are preliminarily administered by pristane (i.p). After 2 weeks, ascites is recovered and purified by DEAE cellulose-column chromatography.

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